

**Amendments to the Specification:**

Please replace the paragraph beginning at page 7, line 23, with the following:

--All technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The present definitions and abbreviations are generally offered to supplement the art-recognized meanings. Generally, the nomenclature used herein and the laboratory procedures organic chemistry, enzyme chemistry and peptide synthesis described below are those well known and commonly employed in the art. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

"AMC," as used herein refers to, 7-amino-4-methyl-coumarin.

"ACC," as used herein refers to, 7-amino-4-carbamoylmethyl-coumarin.

"RFU," as used herein refers to, relative fluorescence units.

"n" and "Nle," as used herein refer to, norleucine.

"PS-SCL," as used herein refers to, positional scanning-synthetic combinatorial library;

"MUGB," as used herein refers to, 4-methylumbelliferyl p-guanidinobenzoate.

"Tris," as used herein refers to, *tris*-(hydroxymethyl)-amino-methane.

"DIC," as used herein refers to, diisopropylcarbodiimide.

"HOBt," as used herein refers to, 1-hydroxybenzotriazole.

"TFA," as used herein refers to, trifluoroacetic acid.

"Fmoc," as used herein refers to, 9-fluorenylmethoxycarbonyl.

"cmk," as used herein refers to, chloromethylketone.

"Nme," as used herein refers to, N-methyl-

"Z," as used herein refers to, benzyloxycarbonyl.

"pNA," as used herein refers to, para-nitroaniline.

"pbf," as used herein refers to, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.

"trt," as used herein refers to, trityl.

"Boc," as used herein refers to, *tert* butoxycarbonyl.

"DMF," as used herein refers to, N,N-dimethylformamide.

"NMP," as used herein refers to, N-methylpyrrolidine.

"TIS," as used herein refers to, triisopropylsilane.

~~"pbf," as used herein refers to, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.~~

"trt," as used herein refers to, trityl.

"HATU," as used herein refers to, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-

tetramethyluronium hexafluorophosphate.--

Please replace the paragraph beginning at page 32, line 32, with the following:

--Tryptase is a potent activator of pro-urokinase plasminogen activator (uPA), the zymogen form of a protease associated with tumor metastasis and invasion. Activation of the plasminogen cascade, resulting in the destruction of extracellular matrix for cellular extravasation and migration, may be a function of tryptase activation of pro-urokinase plasminogen activator at the P4-P1 sequence of Pro-Arg-Phe-Lys (SEQ ID NO:1) (Stack, *et al.*, *Journal of Biological Chemistry* **269**(13): 9416-9419 (1994)). Vasoactive intestinal peptide, a neuropeptide that is implicated in the regulation of vascular permeability, is also cleaved by tryptase, primarily at the Thr-Arg-Leu-Arg (SEQ ID NO:2) sequence (Tam, *et al.*, *Am. J. Respir. Cell Mol. Biol.* **3**: 27-32 (1990)). The G-protein coupled receptor PAR-2 can be cleaved and activated by tryptase at the Ser-Lys-Gly-Arg (SEQ ID NO:3) sequence to drive fibroblast proliferation, whereas the thrombin activated receptor PAR-1 is inactivated by tryptase at the Pro-Asn-Asp-Lys (SEQ ID NO:4) sequence (Molino *et al.*, *Journal of Biological Chemistry* **272**(7): 4043-4049 (1997)). Taken together, this evidence suggests a central role for tryptase in tissue remodeling as a consequence of disease. This is consistent with the profound changes observed in several mast cell-mediated disorders. One hallmark of chronic asthma and other long-term respiratory diseases is fibrosis and thickening of the underlying tissues that could be

the result of tryptase activation of its physiological targets. Similarly, a series of reports during the past year have shown angiogenesis to be associated with mast cell density, tryptase activity and poor prognosis in a variety of cancers (Coussens *et al.*, *Genes and Development* **13**(11): 1382-97 (1999)); Takanami *et al.*, *Cancer* **88**(12): 2686-92 (2000); Toth-Jakatics *et al.*, *Human Pathology* **31**(8): 955-960 (2000); Ribatti *et al.*, *International Journal of Cancer* **85**(2): 171-5 (2000)).--

Please replace the paragraph beginning at page 50, line 20, with the following:

--Factor Xa is an enzyme that plays the critical physiological functions of activating prothrombin and factor VII in the blood coagulation cascade (Davie, E.W., *et al.*, (1991) *Biochemistry* **30**:10363-70). Through profiling with the P1-Arg library, we find factor Xa to show a minor preference for P4-aliphatic amino acids, broad substrate specificity in P3, with the absence of P3-proline activity, and a P2-preference for glycine (**Fig. 3E**). This quantitative information agrees with the qualitative sequences that are efficiently hydrolyzed by factor Xa in a substrate-phage system (2) as well as kinetic studies on tripeptide para-nitroanilide (Cho, K., *et al.*, (1984) *Biochemistry* **23**:644-50) and AMC substrates (Cho, K., *et al.*, (1984) *Biochemistry* **23**:644-50; Lottenberg, R., *et al.*, (1981) *Methods in Enzymology* **80 Pt C**:341-61). Furthermore, the factor Xa P4-P1 cleavage sequence determined here is found in physiologically relevant substrates: the cleavage sequences in prothrombin are Ile-Glu-Gly-Arg (SEQ ID NO:5) and Ile-Asp-Gly-Arg (SEQ ID NO:6); cleavage sequence in factor VII is Pro-Gln-Gly-Arg (SEQ ID NO:7); and the cleavage sequence in the autolysis loop of factor Xa is Glu-Lys-Gly-Arg (SEQ ID NO:8) (Brandstetter, H., *et al.*, (1996) *Journal of Biological Chemistry* **271**:29988-92)).--

Please replace the paragraph beginning at page 53, line 5, with the following:

--To evaluate ACC as a proteolytic leaving group, matched tetrapeptide substrates were made that differed only in the leaving group, ACC or the traditionally used AMC. The two thrombin-susceptible sequences with ACC or AMC, P4-Nle-P3-Thr-P2-Pro-P1-Lys and P4-Leu-P3-Gly-P2-Pro-P1-Lys (SEQ ID NO:9), showed comparable kinetic constants against thrombin (Table II). A significant advantage of ACC substrates over AMC substrates is the ease of synthesizing ACC substrates over AMC substrates. By employing the synthesis methods described, any amino acid ACC substrate can be prepared rapidly with Fmoc-based synthesis protocols.--

Please replace the paragraph beginning at page 54, line 5, with the following:

--The pPIC9-Hu Try (human  $\beta$ I tryptase plasmid) (Niles et al., *Biotechnology and Applied Biochemistry* **28** (Pt 2): 125-31 (1998)) was subjected to site-directed mutagenesis using the GeneEditor™ *in vitro* Site-Directed Mutagenesis System (Promega, Madison WI). The mutant oligonucleotide 5'-GAGGAGCCGGTGAAGGTCTCCAGCCAC-3' (SEQ ID NO:10) was used to introduce a substitution mutation in the DNA coding for amino acid residue 113 (N113K). Full-length nucleic acid sequencing of both strands confirmed the sequence conversion to the  $\beta$ II tryptase isoform.--

Please replace the paragraph beginning at page 60, line 16, with the following:

--The capped peptide backbone of Ac-PRNK-Nme was modelled into the active site of the tryptase structure as follows. The structure of the P1-P3 portion of ovomucoid (complexed to chymotrypsin, PDB code 1cho) was used as a template for the backbone configuration. This portion of the inhibitor was translated into the tryptase active site using least

squares superposition of the protease active site residues His-57, Asp-102, Ser-195, and 214-216 onto the corresponding residues of the tryptase "A" protomer. The peptide sidechains were then truncated at C- $\beta$ , hydrogens and AMBER charges were added (as above) and the configuration of the resultant (Ace-AAA-Nme) peptide was optimized with successive minimizations in the tryptase active site. Using DOCK4.0.1 (Ewing, T. J. A., Makino, S., Skillman, A. G., and Kuntz, I. D. (In Press), the atoms of the scissile amide bond were minimized first, then successive rigid segments of the peptide were added (with torsional angles taken from the ovomucoid inhibitor) alternating with minimization. The minimizations included rigid and flexible degrees of freedom and were performed using the simplex algorithm with up to 500 iterations for each minimization. The DOCK energy scoring, applied to both intermolecular and intramolecular atom pairs, includes the coulombic and van der Waals terms from the AMBER force field (Ewing, *supra*; Weiner *et al.*, *Journal of Computational Chemistry* 7(2): 230-252 (1986)). An interatomic cutoff of 25 Å and  $\epsilon = 4r$ . The peptide side chains (PRNK; SEQ ID NO:11) were then added, and the conformation of the P1-P3 side chains and the P4 proline were modelled with DOCK4.0. Finally, 10 independent minimizations were carried out, and the lowest-energy configuration was retained.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 4, at the end of the application.